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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

02078228.0

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Synthesis, radiosynthesis and clinical application of [18F] labeled L-and/or D-.-(o,m,p) fluoromethylphenyl-.-aminopropionic acid (L-/D-(o,m,p)F-methyl-phenylalanine),L-and/or D-.-(o,m,p) fluoroethylphenyl-.-aminopropionic acid (L-/D-(o,m,p)F-ethyl-phenylalanine,L-/D-2-amino-3-(2-fluoroalkyl-4-hydroxy-phenyl) propianoic acid [alkyl=methyl or ethyl] (L-/D-2-fluoroalkyl-tyrosine), L-and/or D-18F-Leucine and 18F-Isoleucine

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
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AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LU MC NL PT SE SK TR

Title of the Invention :

Synthesis, radiosynthesis and clinical application of [^{18}F] labeled L- and/or D-β-(o,m,p) fluoromethylphenyl-α-Aminopropionic acid (L-/D-(o,m,p)F-methyl-phenylalanine), L- and/or D-β-(o,m,p) fluoroethylphenyl-α-Aminopropionic acid (L-/D-(o,m,p)F-ethyl-phenylalanine),L-/D-2-Amino-3-(2- fluoroalkyl-4-hydroxy-phenyl) propianoic acid [alkyl = methyl or ethyl] (L-/D-2-fluoroalkyl-tyrosine) , L- and/or D-^{18}F-Leucine and ^{18}F-Isoleucine.

Brief Description of the Invention :

The present invention relates to synthetic amino acid analogs labeled with F-18, to a method of preparing these compounds, to an automated system for preparing these compounds, to a pharmaceutical composition comprising these compounds and to the use of this composition for diagnosis with Emission Computered Tomography. The invention also relates to the application of L- and or D- (L/D)-^{18}F- fluoromethyl- phenylalanine and L-/D-^{18}F-fluoroethyl- phenylalanine analogues, L-/D-2-^{18}F-fluoromethyl-tyrosine and L-/D-2-^{18}F-fluoroethyl-tyrosine as tumor specific tracers for diagnosis with Positron Emission Tomography. The substitution of an alkyl group, provided with an appropriate leaving group on the phenyl ring of phenylalanine, and introduction of the ^{18}F atom by aliphatic nucleophilic substitution. Synthesis of the precursor molecules allowing to obtain the required ^{18}F-alkyl-phenylalanine and ^{18}F-alkyl-tyrosine analogues. The application of L- and/or D-^{18}F-Leucine and L-/D-^{18}F-Isoleucine as tumor specific tracer for diagnosis with Positron Emission Tomography.

The invention also relates to the synthesis of the precursor molecules allowing to obtain the required ^{18}F-substituted Leucine and Isoleucine analogues by the substitution of an appropriate leaving group on the alkyl part of Leucine and Isoleucine. The automated radiosynthesis of mentioned compounds.

Purpose of Invention :

Whatever the new approaches for therapy of cancers will be in the future, an accurate and specific non-invasive diagnosis on bio-molecular level of tumors and metastases remain of primary importance. Transformation of normal cells into malignant cells is caused by changes in the genetic material, resulting in subtile but fundamental metabolic changes like increased glucose metabolism and increased amino acid uptake and metabolism. These

changes in the metabolic phenotype permit the *in-vivo* study of tumors using radioactive labeled tracers coupled to SPECT (Single Photon Emission Computed Tomography) or PET (Positron Emission Tomography). PET linked coincidence acquisition allows a better resolution and quantification than SPECT, needed for tumor tracing and dimensioning.
Currently, the use of ^{18}F-FDG (Fluoro-deoxyglucose) and PET is the most important technique in nuclear medicine for the study of oncological patients. Although this method is very sensitive, it has two major limitations, namely an avid accumulation in inflammatory lesions and high uptake in the brain, jeopardizing the diagnosis of brain tumors.
It was shown that the use of radioactive amino acids for SPECT and PET could overcome these shortcomings for the larger part[1]. In the late 80's, several ^{11}C-labelled amino acids like methionine and tyrosine, as well as 2-^{18}F-tyrosine (2-^{18}F-Tyr) of high specific activity were used for PET studies[2]. At that time it was believed that a high specific activity was required and that for tumor specification the labeled amino acid had to be involved in a high rate protein incorporation. None of these amino acids has meanwhile been introduced into routine clinical PET because of the short half life and insufficient in vivo stability of C-11 or complicated radiochemical synthesis resulting in insufficient yield (2-^{18}F-Tyr).
About the same time, L-3-^{123}I-alpha-methyl-tyrosine (3-^{123}I-IMT) was introduced as a SECT tracer for brain tumors[3] and is used until now also for other tumors like sarcoma and lymphoma. The uptake of this tracer in tumors occurs for the larger part by the L transport system[4,5] and the increased accumulation is mainly determined by strongly increased amino acid transport activity rather than incorporation into proteins. A major drawback limiting the applicability of this tracer is the high renal accumulation.
O-(2-^{18}F-ethyl)-tyrosine (FET) and ^{18}F- alpha -methyl-tyrosine were proposed in 1999 as potential PET tracers[6]. FET showed the same uptake properties as IMT. The preparation of these tracers still requires complicated and time consuming synthetic steps and HPLC steps limiting the overall radiochemical yield.

We recently developed two new potential SPECT tracers: 2-^{123}I-tyrosine (2-^{123}I-Tyr)[7,8,9] and 2-^{123}I-phenylalanine[10,11,12]. When evaluated in vivo in R1M tumor (rabdomyo-sarcoma)-bearing rats, these tracers showed high uptake in the tumors (comparable with IMT) while no renal accumulation (10 times less activity in the kidneys than IMT) or high brain uptake was observed.

Our kinetic studies also revealed that the uptake of radioactive amino acid reflected the amounts of amino acids in the tumor as compared to the blood pool compartment and that no high specific activity is required for the tracer.

Leucine (Leu) and isoleucine (Ile) : preliminary uptake experiments in R1M cells *in-vitro* in a buffer simulating *in-vivo* conditions, showed for ^{3}H-leucine and ^{3}H-isoleucine results comparable with ^{3}H-Tyr and ^{3}H-Phe. As it is supposed that aliphatic-substituted F hardly changes the pharmacology, this makes these aliphatic amino acids promising candidates for radio-fluorination.

It was shown that a ^{18}F-labelled amino acid as tumor tracer shows higher tumor specificity as compared to FDG and is better suited as brain tracers.
The fact that within toxicity limits neither high specific activity nor non-carrier added preparation of the ^{18}F-tracer is required, should allow electrophilic radio-fluorination making use of [^{18}F]-F_2. The radioisotope production yield with the currently available F_2 - targets is limited and even with an almost quantitative labeling yield, amounts comparable with those of the ^{18}F-FDG production are far from being reached and does not allow routine multi patient PET diagnosis.

Detailed Description of the invention :

The results obtained with ^{18}F-FET, our own results with 2-I-Phe and 2-I-Tyr and the results described for 4-I-Phe[13] show that the aromatic amino acid properties are conserved after substitution of an O-ethyl group and of even a voluminous iodine atom.
This invention shows a new approach, the ^{18}F-alkyl-phenyl structure in Phe and Tyr : ortho, meta or para ^{18}F-CH_2-Phe or ^{18}F-CH_2-CH_2-Phe and 2-^{18}F-CH_2-Tyr or 2-^{18}F-CH_2-CH_2-Tyr . This reduces the labelling chemistry to direct conventional nucleophilic aliphatic substitution on the alkylphenylic side branch of the L-amino acid coupled to an automated mini-column purification and recovery. In this approach cumbersome stereospecific synthesis is not required.
^{18}F-Leu and Ile: the same strategy will be followed for the radio-fluorination of the aliphatic amino acids Leu and Ile.

Synthesis of precursor molecules :

L-/D-2-Tos (Trif)-CH_2-Phe and any suitable leaving group as described in literature : L-2-CH_3-Phe is commercially available. After stereospecific chromatographic separation L-2-CH_3-Phe will be protected by esterification (^{t}But) and as N-Boc or N-trityl. Bromine is introduced by radical attack. A tosyl (Tos) or a triflate (Trif) group and any suitable

leaving group as described in literature is introduced by nucleophilic exchange. After purification, the compound is stored under nitrogen.

As L-/D-2-Br-Phe is commercially available, this precursor molecule can also be obtained by a Wurtz-Fittig reaction, using dibromomethane and then applying the same pathways as described above.

L-2-Tos(Trif)-CH_2-Tyr : CH_3O-L-2-I-Tyr is commercially available and is an adequate precursor for the Wurtz-Fittg pathway mentioned above.

For the synthesis of L-/D-2-(Trif,Tos)ethyl-Phe, L-/D-4-(Trif,Tos)ethyl-Phe, L-/D-2-(Tros, Trif) methyl-Tyr and L-/D-2-(Tros,Trif) ethyl-Tyr and any suitable leaving group as described in literature, the same strategies are followed.

For Leu and Ile a place specific bromination is applied, followed by introduction of the appropriate leaving group.

Example of in vitro affinity for cancer cells :

Determination of the affinity of 2-methyl-phenylallanine and 2-fluoromethyl-phenylalanine for uptake and for the L-transporter system in cancer cells (rat rabdomyosarcoma cells), ^{3}H-phenylalanine uptake after 15 min incubation in HEPES buffer of pH 7.4 containing appropriate amounts of phenylalanine and of 2-methyl-phenylalanine as inhibitor was measured.

The uptake followed the typical Michaelis-Menten relation allowing to draw Lineweaver-Burk (Fig.1) and Eady-Hofstee (Fig.2) plots. The double reciprocal plots in Fig.1 with a common intercept almost on the 1/uptake axis shows that the largest part of the inhibition is competitive with the phenylalanine uptake and uses the same LAT transporter system. This is also shown in Fig.2. A slight deviation of the Y-axis in both cases can be due to a small amount of ^{3}H-phenylalanine already incorporated in the proteins. Comparable results are obtained with the D-alkyl-phenylalanine analogs.

A mean K_i value of 76 µM was obtained for L-/D-2-methyl-phenylalanine as compared to the calculated apparent K_m value of 95 µM for L- and D-phenylalanine. This shows that the affinity of the L- as well as the D-methyl analogue is comparable to that of the natural phenylalanine

Radiochemical synthesis :

L-/D-^{18}F-R-Phe analogues (R = methyl or ethyl): nucleophilic exchange of ^{18}F on L-/D-2-TrifR-Phe or L-/D-2-TosR-Phe in an AcN/TBA/HCO_3 or AcN/K_{222}/CO_3^{2-} mixture at 85°C

during 5 minutes. Two pathways are possible: 1° de-esterification and de-protection in solution followed by mini-column purification or straightforward de-protection on column followed by purification.

L-/D-^{18}F-Leu and L-/D-^{18}F-Ile: an analogous radiochemistry will be applied.

This radiochemistry is automated and adapted to PC steered Synthesis Box preparation.

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CLAIMS

1. Synthetic amino acid analogs labeled with F-18 as disclosed in the description.
2. Method for preparing the compounds of claim 1, as described in the dscription.
3. Automated system for preparing the compounds of claim 1, as described in the dscription.
4. Pharmaceutical composition comprising the compounds as claimed in claim 1.
5. Use of the composition as claimed in claim 4 for diagnosis.

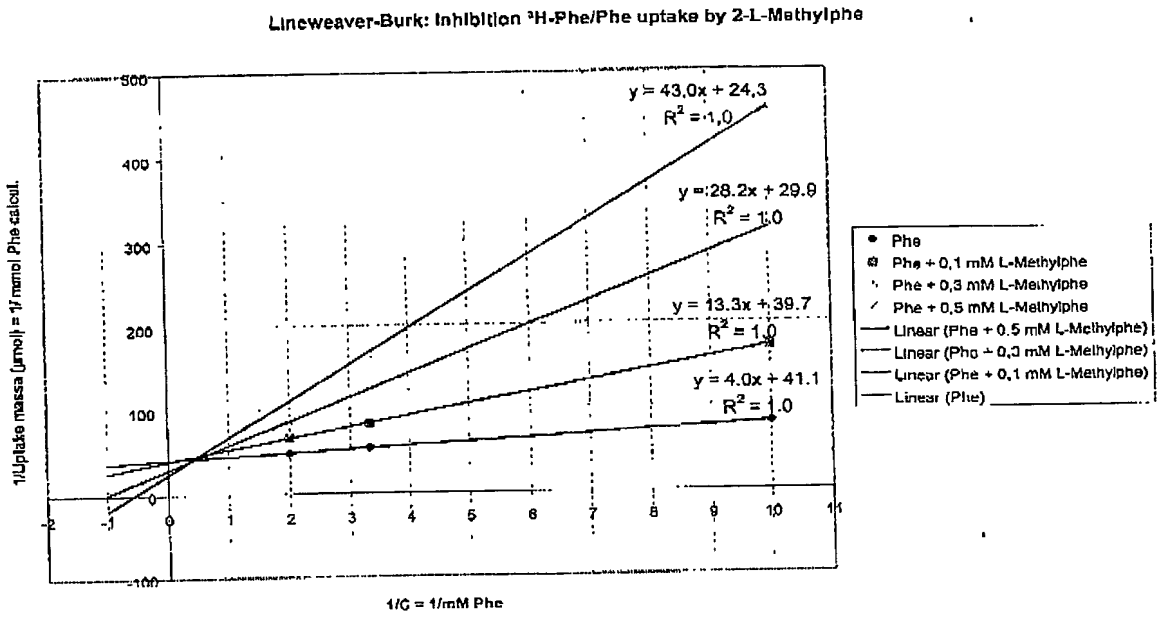
Lineweaver-Burk: Inhibition ³H-Phe/Phe uptake by 2-L-Methylphe
y = 43.0x + 24.3
R² = 1.0
y = 28.2x + 29.9
R² = 1.0
y = 13.3x + 39.7
R² = 1.0
y = 4.0x + 41.1
R² = 1.0
1/Uptake massa (µmol) = 1/ mmol Phe calcul.
1/C = 1/mM Phe
Phe
Phe + 0,1 mM L-Methylphe
Phe + 0,3 mM L-Methylphe
Phe + 0,5 mM L-Methylphe
Linear (Phe + 0.5 mM L-Methylphe)
Linear (Phe + 0,3 mM L-Methylphe)
Linear (Phe + 0,1 mM L-Methylphe)
Linear (Phe)

Fig 1

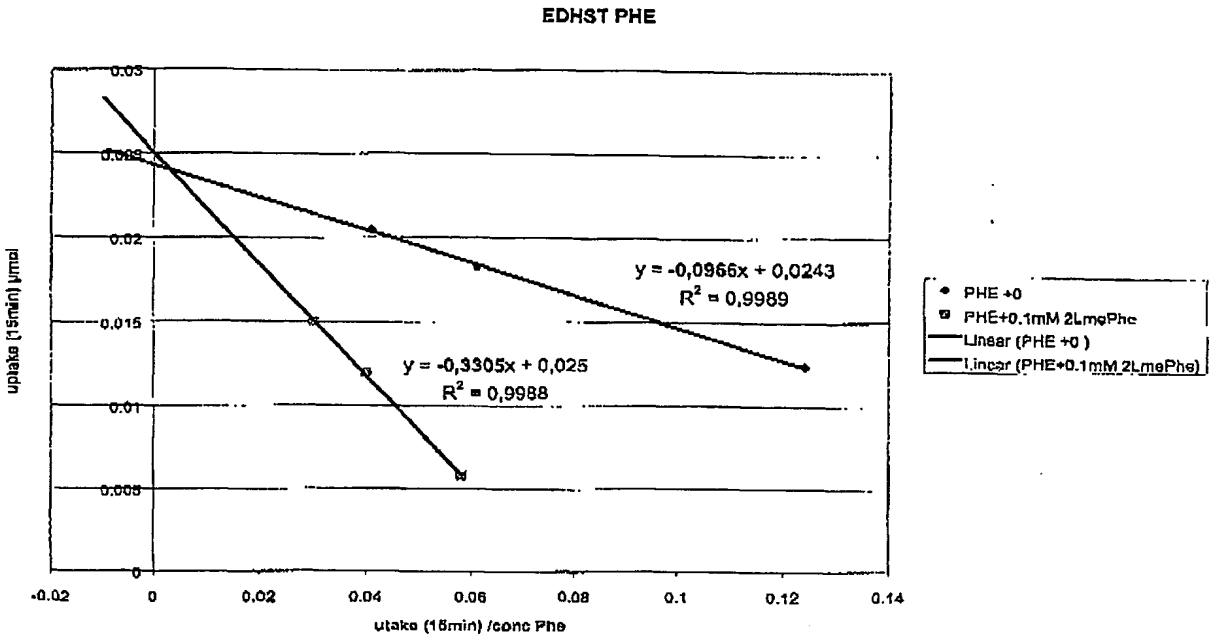
EDHST PHE
uptake (15min) µmol
0.03
0.025
0.02
0.015
0.01
0.005
0
-0.02
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
uptake (15min) /conc Phe
y = -0,0966x + 0,0243
R² = 0,9989
y = -0,3305x + 0,025
R² = 0,9988
PHE +0
PHE+0.1mM 2LmePhe
Linear (PHE +0)
Linear (PHE+0.1mM 2LmePhe)

Fig 2